



# EliGene® *Chlamydomphila pneumoniae* UNI

**REF** 90057-UNI (for 50 samples)

## Kit components:

5 x 150 µl *C. pneumoniae* UNI Mix  
2 x 50 µl PC DNA *C. pneumoniae*  
5 x 200 µl IC DNA 01  
1 x 20 µl Eli Blocker  
1 x 20 µl ROX dye  
1 x Instruction for Use

## Storage and shelf life after first opening:

All components of the kit must be transported and stored at -20 °C. Kit and remaining MasterMixes must be stored at -20 °C in a dark.

## Intended use

EliGene® *Chlamydomphila pneumoniae* UNI is intended for DNA diagnostics of pathogenic species *Chlamydomphila pneumoniae* from isolated DNA sample.

## Principle of the method

This diagnostic kit is based on RealTime PCR method using Molecular Beacons hybridization probes. In this kit for the detection of *Chlamydomphila pneumoniae* and internal control primers and Molecular Beacons labeled probes (FAM and HEX) are used.

## Introduction

*Chlamydomphila pneumoniae* is an obligate intracellular pathogen. It is a member of the *Chlamydiae*, a diverse range of obligate intracellular bacteria that includes parasites of amoebae, fish, reptiles, mammals, and humans. Today *C. pneumoniae* is associated with a remarkably wide range of chronic diseases as meningoencephalitis, arthritis or myocarditis and it is the second most often cause of community-acquired pneumonia. *C. pneumoniae* spreads by drop infection but in the form of infection elementary bodies (EB) resistant to environmental stresses, it can persist on the surfaces or be spreaded by the aerosol. During the life about 80% of population is infected by *C. pneumoniae* however in majority of cases the infection is asymptomatic. Some forms of pneumonia can be very serious but in these cases is not clear if *C. pneumoniae* is not only co-pathogen.

## Primary sample collection, handling and storage

Clinical material:	Recommended DNA isolation procedure:
Throat swabs, Nasopharyngeal aspirates,	Manual: EliGene® Urine Isolation Kit
Sputum	Automatic: ZEPHYRUS Magneto (ELISABETH PHARMACON)



**WARNING:** To keep the sensitivity of the test we recommended strictly follows the pre-analytical procedures mentioned in this instruction. Specially do not change the recommended procedures of isolation as for stated amount of specimen, centrifugation force, etc.

#### **Throat swabs:**

These specimens should be collected before the treatment by antibiotics according to standard protocol. Specimens in cultivation medium can be stored at 4°C up to seven days.

Recommended procedure for swab specimens processing

1. Just before the DNA isolation immerse throat swabs in 1 ml of PBS. After agitating the swabs for 3 min, open the tube and by pressing the swab along the tubes wall press out the all solution from the swab. Discard the swab.
2. Centrifuge the tube for 15 minutes at 6000 x g or 10 minutes at 10 000 x g.

#### *Manual isolation:*

1. Aspirate supernatant. Resuspend pellet in 180 µl of molecular grade water with 200 µl of MI3 solution from EliGene® Urine Isolation Kit, and add 10 µl of Proteinase K and 20 µl of Internal Control DNA 01 (IC DNA 01). Vortex for 15 seconds and continues according to instructions in standard protocol in EliGene® Urine Isolation Kit.
2. Elution is done to 50 µl of elution buffer. Use DNA immediately after the isolation for the analysis or store DNA hours to one week at 4°C or freeze DNA at -20°C for longer period than one week.

#### *Automatic isolation:*

1. Open the tubes and carefully aspirate all supernatant. Re-suspend pellets in 180 µl of PBS by vortexing and add 20 µl of Internal control IC DNA 01.
2. Isolate DNA from the sample by using MAGNETO **BodyFluid DNA/RNA isolation kit** according to protocol for Plasma samples with Elution to 50 µl of Elution buffer.

#### **Nasopharyngeal aspirates (NPAs), sputum:**

Homogenize NPAs and sputum specimens by adding an equal volume of a mucolytic agent (dithiotreitol, final concentration 4–6 mM). Centrifuge the specimen at 6000 x g for 15 minutes before DNA isolation.

#### *Manual isolation:*

1. Aspirate supernatant. Resuspend pellet in 180 µl of molecular grade water with 200 µl of MI3 solution from EliGene® Urine Isolation Kit, and add 10 µl of Proteinase K and 20 µl of Internal Control DNA 01 (IC DNA 01). Vortex for 15 seconds. Continue according to instructions in standard protocol in EliGene® Urine Isolation Kit.
2. Elution is done to 50 µl of elution buffer. Isolated DNA use immediately for the detection or store it hours to one week at 4 °C or freeze DNA at -20 °C for longer period than one week.

#### *Automatic isolation:*

1. Open the tubes and carefully aspirate all supernatant re-suspend pellets in 200 µl of PBS by vortexing and add 20 µl of Internal control IC DNA 01.
2. Isolate DNA from the sample by using MAGNETO **BodyFluid DNA/RNA isolation kit** according to protocol for Plasma samples with Elution to 50 µl of Elution buffer.



### Additional required equipment

- Automatic pipette 5-20 µl and sterile tips with filter DNA RNA free, DNase-, RNase- free (we recommended plastic with CE certificate for diagnostic purposes).
- Sterile stand DNA-, RNA- free, DNase-, RNase- free.
- Equipment for RealTime PCR – the kit is designed for RealTime Systems ABI7500 (Applied Biosystems), LightCycler 2.0, LightCycler 480, LightCycler Nano (Roche), RotorGene 6000 or RotorGene Q (Qiagen).
- Sterile plastic (strips, plates, tubes) DNase-, RNase- free compatible with given RealTime PCR system.
- Lab safety gloves

### Configuration of Real Time instrument

- For *Chlamydomydia pneumoniae* detection the probe labeled with FAM is used (exc. 494 nm – em. 518 nm)
- For Internal control the probe labeled with HEX is used (exc. 520 nm – em. 548 nm)

#### LightCycler® 2.0 (Roche):

For reaction use glass capillaries only.

**Before the using of EliGene® LC kits it is necessary to perform spectral calibration with EliGene® LC Spectral Calibration Kit 2 (Cat. No. 90099-SC2)! Once the spectral calibration was done, it is valid until the instrument is upgrading or optic is changed.**

#### Set up the following temperature profile:

Step 1 - Analysis mode "None", 1 Cycle

95°C	3 min	Ramp rate (20°C/s)	Acquisition mode "None"
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Step 2 - Analysis mode "Quantification", 50 Cycles

95°C	5 s	Ramp rate (20°C/s)	Acquisition mode "None"
55°C	20 s	Ramp rate (5°C/s)	Acquisition mode "Single"
72°C	10 s	Ramp rate (20°C/s)	Acquisition mode "None"

The complete temperature profile can be up-loaded from Run Template "CPNEU\_UNILC2\_v00.ix0". The Run Template can be imported to the software in menu "Navigator" by clicking to icon "Import" from the CD included in the kit.

#### LightCycler® 480 (Roche):

For reaction use white plates only. The usage of natural plates can lead to decreased sensitivity of the kit. Do not reuse plates; the contamination of your laboratory could occur during the manipulation with plates.

In option Detection format choose "Dual Color Hydrolysis probe".



**Set up the following temperature profile:**

*Step 1 - Analysis mode "None", 1 Cycle*

95°C	3 min	Ramp rate (4.4°C/s)	Acquisition mode "None"
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*Step 2 - Analysis mode "Quantification", 50 Cycles*

95°C	5 s	Ramp rate (4.4°C/s)	Acquisition mode "None"
55°C	20 s	Ramp rate (2.2°C/s)	Acquisition mode "Single"
72°C	10 s	Ramp rate (4.4°C/s)	Acquisition mode "None"

*Step 3 - Analysis mode "None", 1 Cycle*

40°C	1 min	Ramp rate (2.2°C/s)	Acquisition mode "None"
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The complete temperature profile can be up-loaded from Run Template "CPNEU\_UNILC480\_v00.ix0". The Run Template can be imported to the software in menu "Navigator" by clicking to icon "Import" from the CD included in the kit.

**LightCycler® Nano (Roche):**

For reaction use clear strips only.

In "Run Settings" menu choose "Hydrolysis Probes" option and "High Quality" option.

**In "Profile menu" set up the following temperature profile:**

*Step 1 - Hold*

95°C	3 min	Ramp rate (5°C/s)	
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*Step 2 – 3-Step Amplification, 50 cycles*

95°C	5 s	Ramp rate (5°C/s)	
55°C	20 s	Ramp rate (4°C/s)	"Acquire" signal
72°C	10 s	Ramp rate (5°C/s)	

*Step 3 - Hold*

40°C	1 min	Ramp rate (4°C/s)	
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In "Samples" menu click in window "Targets" (upper right window) on icon "+" and choose FAM dye as "Target 1". Then click once again on icon "+" and choose HEX dye as "Target 2". In window "Samples" (upper left window) click on icon "+" and add your samples. Then assign the samples with positions and Targets FAM and HEX as an Unknown sample (Samples) or Standard.

The complete temperature profile can be up-loaded from Run Template "CPNEU\_UNILCNANO\_v00.ppf". The Run Template can be copied from the CD included in the kit.



### **RealTime System ABI7500FAST (Applied Biosystems):**

Use the program module for absolute quantification (Plate Type "Quantitation-Standard Curve" experiment for ABI 7500FAST). In case of ABI7500FAST use "7500 (96wels)" instrument type.

#### **Set up the following temperature profile:**

##### *Holding stage*

95°C                    3 min

##### *Cycling stage – 50 cycles*

95°C                    5 s

55°C                    20 s      Data collection ON

72°C                    10 s

*Collect emission signal at the second step – 55°C.*

The complete temperature profile can be up-loaded from Run Template "CPNEU\_UNIABI\_v00.edt". The Run Template can be copied from the CD included in the kit.

### **RotorGene 6000 or Q (Qiagen):**

In the "New Run" window choose "Three Step" run

Choose the appropriate "Rotor Type" and click "Next".

#### **Set up the following temperature profile:**

##### *Holding stage*

95°C                    3 min

##### *Cycling stage – 50 cycles*

95°C                    5 s

55°C                    20 s      Acquiring in channels "Green" and "Yellow"

72°C                    10 s

The complete temperature profile can be up-loaded from Run Template "CPNEU\_UNIRG\_v00.ret". The Run Template can be copied from the CD included in the kit.

### **Reagent preparation**

- To avoid the contamination keep all tubes closed and follow the instructions.
- Before the usage, all reagents must be completely thawed, briefly mix on vortex and shortly spin.
- In the step of Proteinase K addition of Isolation protocol add 20 µl of Internal Control DNA 01 (IC DNA 01) to isolated sample. In no case add the internal control to isolated DNA just before the analysis.

### **Preparation of Reaction Mix except LightCycler® 2.0 and ABI instrumets**

1. Detection: Take one microtube with C. pneumoniae UNI Mix. After the thawing pipette 15 µl of the Mix to amplification microtubes or plate and add 5 µl of isolated DNA. During the pipetting of samples be careful to avoid cross-contamination of samples. If you do not use all the volume of MasterMixes, freeze it and store at -20 °C in a dark. Do not freeze tubes with MasterMixes repeatedly. Under these conditions it is stable at least for 14 days.



2. Positive control: Take one microtube with C. pneumoniae UNI Mix. After the thawing pipette 15 µl of Mix to amplification microtube or plate and add 5 µl of PC DNA C. pneumoniae. During the pipetting of positive control be careful to avoid contamination of the other samples.

Insert the micro tubes or plate with samples to the RealTime PCR instrument and run the program according to chapter "Configuration of Real Time instrument" above.

### **Preparation of Reaction Mix for LightCycler® 2.0**

1. Preparation of MasterMix: Prepare the MasterMixes by mixing EliBlocker solution with C. pneumoniae UNI Mix. Take one tube of C. pneumoniae UNI Mix and thaw the content at the room temperature. Immediately after the thawing spin shortly the microtubes and add 3.0 µl of EliBlocker solution to microtube with C. pneumoniae UNI Mix. Mix gently by pipetting up and down and shortly spin.
2. Detection: Take one microtube with C. pneumoniae MasterMix. Pipette 15 µl of the Mix to amplification microtube or plate and add 5 µl of isolated DNA. During the pipetting of samples be careful to avoid cross-contamination of samples. If you do not use all the volume of MasterMixes, freeze it and store at -20 °C in a dark. Do not freeze tubes with MasterMixes repeatedly. Under these conditions it is stable at least for 14 days.
3. Positive control: Take one microtube with M. pneumoniae MasterMix. Pipette 15 µl of the Mix to amplification microtube or plate and add 5 µl of PC DNA C. pneumoniae. During the pipetting of positive control be careful to avoid contamination of the other samples.

Insert the micro tubes or plate with samples to the RealTime PCR instrument and run the program according to chapter "Configuration of Real Time instrument" above.

### **Preparation of Reaction Mix for ABI instruments**

1. Preparation of MasterMix: Prepare the MasterMixes by mixing ROX dye solution with C. pneumoniae UNI Mix. Take one tube of C. pneumoniae UNI Mix and thaw the content at the room temperature. Immediately after the thawing spin shortly the microtube and add 3.0 µl of ROX dye solution to microtube with C. pneumoniae UNI Mix. Mix gently by pipetting up and down and shortly spin.
2. Detection: Take one microtube with C. pneumoniae MasterMix. Pipette 15 µl of the Mix to amplification microtubes or plate and add 5 µl of isolated DNA. During the pipetting of samples be careful to avoid cross-contamination of samples. If you do not use all the volume of MasterMixes, freeze it and store at -20 °C in a dark. Do not freeze tubes with MasterMixes repeatedly. Under these conditions it is stable at least for 14 days.
3. Positive control: Take one microtube with C. pneumoniae MasterMix. Pipette 15 µl of each Mix to individual amplification microtubes or plate and add 5 µl of PC DNA C. pneumoniae. During the pipetting of positive control be careful to avoid contamination of the other samples.

Insert the micro tubes or plate with samples to the RealTime PCR instrument and run the program according to chapter "Configuration of Real Time instrument" above.

### **Result viewing**

#### **LightCycler® 2.0 (Roche):**

Analyze real-time PCR results in channels 530 and 560 (LightCycler® Software 4.X) respectively, using the Qualitative detection module of the LightCycler® Analysis Software.



Positive result for *Chlamydomphila pneumoniae*: The positive result is characterized by amplification and growth of signal in FAM channel (530) of C. pneumoniae UNI Mix reaction. In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (560) in C. pneumoniae UNI Mix.

Cp value corresponds to the quantity of positive result. The value of "Score" must be higher than 4.5.

### LightCycler® 480 (Roche):

In "Sample Editor" menu choose "Abs Quant" workflow.

In menu "Analysis" choose "Abs Quant/2nd Derivative Max" option.

In Analysis window click to "Color Comp" icon and choose Universal CC FAM (510)-VIC (580) calibration. Analyze results by clicking to icon "Calculate".

Positive result for *Chlamydomphila pneumoniae*: The positive result is characterized by amplification and growth of signal in FAM channel (465-510) of C. pneumoniae UNI Mix reaction. In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (533-580) in C. pneumoniae UNI Mix.

The values of Concentration correspond to the quantity of positive result; "Negative" means negative result. Positive result is characterized by increasing of fluorescence signal in selected channel.

### LightCycler® Nano (Roche):

In "Analysis" menu click in window "Select Analysis" on icon "+" and choose "Automatic Quantification".

Positive result for *Chlamydomphila pneumoniae*: The positive result is characterized by amplification and growth of signal in FAM channel (510-528) of C. pneumoniae UNI Mix reaction. In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (530-548) in C. pneumoniae UNI Mix.

The values of Concentration correspond to the quantity of positive result; "Negative" means negative result. Positive result is characterized by increasing of fluorescence signal in selected channel.

### RealTime System 7500FAST (Applied Biosystems):

In "Analysis Settings" choose "Automatic Treshold" and "Automatic Baseline" option and analyze results.

Positive result for *Chlamydomphila pneumoniae*: The positive result is characterized by amplification and growth of signal in FAM channel (em. 518 nm) of C. pneumoniae UNI Mix reaction. In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (em. 548 nm) in C. pneumoniae UNI Mix.

The values of Qty correspond to the quantity of positive result, "result undet." means negative result. Positive result is characterized by increasing of fluorescence signal in given channel.



### **RotorGene 6000 or Q (Qiagen)- version 1.7 and higher:**

Click to “Analysis” icon in the menu and choose Analysis option “Quantitation”. In “Quantitation Analysis” window choose “Dynamic Tube” and “Slope Correct” option.

Positive result for *Chlamydomphila pneumoniae*: The positive result is characterized by amplification and growth of signal in FAM channel (Green) of C. pneumoniae UNI Mix reaction. In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (Yellow) in C. pneumoniae UNI Mix.

The values of “Calc. conc.” correspond to the quantity of positive result; “Negative” means negative result. Positive result is characterized by increasing of fluorescence signal in given channel.

### **Interpretation of results**

#### **Negative result:**

If the increasing of amplification signal in FAM channel of C. pneumoniae UNI Mix does not appear before cycle number 45, the result of test should be interpreted as probably *Chlamydomphila pneumoniae* DNA negative or with concentration of DNA below the detection limit of this kit (10 genomic DNA/reaction). The signal for Internal Control must be positive – see article Quality control. This result does not exclude the occurrence of *Chlamydomphila pneumoniae* infection because results of this test are dependent on proper sample collection and elaboration. Results are also dependent on enough quantity of analyzed *Chlamydomphila pneumoniae* DNA.

#### **Positive result:**

Amplification signal in FAM channel (465-510) of C. pneumoniae UNI Mix appears before cycle number 45. *Chlamydomphila pneumoniae* DNA was detected in the sample. The sample is *Chlamydomphila pneumoniae* DNA positive.

**WARNING:** The contamination in laboratory space is also possible. Use separate pipette for Master mixes, separate pipette for positive controls and separate pipette for samples. Follow all recommendations for laboratories of DNA analyses.

#### **Inhibited sample:**

In the case that increasing of amplification signal specific for *Chlamydomphila pneumoniae* in FAM channel and also increasing of amplification signal specific for internal control in HEX channel is not observed, it is necessary to repeat the analysis. The best, there is to use DNA samples prepared by new DNA isolation.

### **Control procedure**

EliGene® *Chlamydomphila pneumoniae* UNI Kit involves internal control (IC DNA 01) and positive control (PC DNA C. pneumoniae). Internal isolation control follows the quality of DNA isolation and detects mistakes in the isolation process. It detects the occurrence of an inhibition of amplification process. In the case that the sample is *Chlamydomphila pneumoniae* DNA negative, the Cp of internal control must be Cp < 35. In the case of strongly positive samples usually the internal control amplification is not detected.

Positive control follows the proper function of MasterMix. Minimal Cp of positive control must be 35 or less. The Cp higher than 35 for positive control can't be accepted and DNA detection must be repeated with new sample. In the case of repeatedly higher Cp contact manufacturer ELISABETH PHARMACON.

Use negative control for each run. As negative control use the water for molecular biology used in your laboratory. For negative control use the pipette for DNA samples.





### Reference material:

To monitor the all examination process covering DNA isolation and RealTime PCR detection is possible to use specimen positive for *Chlamydomphila pneumoniae*. The commercial positive material is not available.

### Troubleshooting:

1. If there is no amplification of Internal Control, there is some problem in the isolation of DNA or the kit is after the expiration date or there is RealTime instrument breakdown.
2. If there is no amplification of Positive Control, the kit is after the expiration date or there is RealTime instrument breakdown.

## Performance characteristics

### Analytical performance characteristics:

Kit EliGene® *Chlamydomphila pneumoniae* UNI has a very high sensitivity - detects 10 genomic or plasmid DNA added to the amplification mix. Analytical sensitivity of the test was verified as follows. The plasmid with *Mycoplasma* sequence was prepared and diluted to desired concentration of target sequence. The addition of human DNA to sample in common concentration has no effect to sensitivity of the method.

**Analytical sensitivity** is 10 copies of *Chlamydomphila pneumoniae* in reaction mix.

**Analytical specificity** of method is 100%. Analytical specificity of method was verified by searching the DNA database NCBI (GenBank, <http://www.ncbi.nlm.nih.gov/>) for the sequences of primers and probes and by addition of human DNA to mastermix. 50 different samples did not give false positive result. False positive result was not observed after the addition of DNA from these organisms *B. burgdorferi sensu lato*, *M. tuberculosis*, *M. bovis*, *M. cansasii*, *M. xenopii*, *M. avium*, *M. marinum*, *Mycoplasma hominis* and *genitalium* *Lactobacillus*, *Enterococcus faecalis*, genus *Pseudomonas*, *E. coli*, *A. niger*, *C. albicans*, *S. aureus*, *S. agalactiae*, *Ch. trachomatis*, *U. urealyticum*, Adenovirus, HBV, EBV, CMV, HSV1, HSV2, VZV.

**Clinical specificity** was tested on human blood and serum samples. 50 samples of different human DNA isolated from the blood and serum did not give false positive result.

### Diagnostic performance characteristics:

Within the frame of testing the functional characteristics of EliGene® *Chlamydomphila pneumoniae* UNI kit overall 50 clinical specimens were analyzed. From these 50 clinical specimens were 14 positive. EliGene® *Chlamydomphila pneumoniae* UNI kit analyzed all 14 samples as positive. Totally 36 clinical specimens were properly by EliGene® *Chlamydomphila pneumoniae* UNI kit analyzed as negative.

Sensitivity: 100%

Specificity: 100%

### Measuring interval

The kit enables the detection of  $10^1$  -  $10^8$  of *Chlamydomphila pneumoniae* DNA molecules in reaction Mix.

### Internal control of quality

As an internal control of quality the Internal Control for checking the process of DNA isolation together with Positive Control for functional control of MasterMix and as a reference sample is used.

### Limitation of the examination procedure

The sensitivity of kit depends on handling with specimen (isolation of DNA). It is strictly recommended to use isolation kits and procedures mentioned above.



Negative result does not exclude the occurrence of *Chlamydomphila pneumoniae* infection because results of this test are dependent on proper sample collection and elaboration. Results are also dependent on enough quantity of analyzed *Chlamydomphila pneumoniae* DNA.

### Biological reference intervals

Not applicable information for this kit.

### Warning

After mixing MasterMix is stable for 2 weeks at -20 °C. Do not freeze tubes with MasterMix repeatedly! Do not mix components of the kits of different lots.

### Warnings and general precautions

**This kit is intended for *in vitro* use only.**

- Handle and dispose of all biological samples as if they were capable of transmitting infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying. The materials that come into contact with biological samples must be treated with 3% sodium hypochlorite for at least 30 minutes or autoclaved at 121 °C for one hour before disposal.
- Handle and dispose of all reagents and all assay materials as if they were capable of transmitting infective agents. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be treated and disposed of in compliance with the appropriate safety standards. Disposable combustible materials must be incinerated. Liquid waste containing acids or bases must be neutralized before disposal.
- Wear suitable protective clothing and gloves and protect eyes/face.
- Never pipette solutions by mouth.
- Do not eat, drink, smoke or apply cosmetic products in the work areas.
- Wash hands carefully after handling samples and reagents.
- Dispose of leftover reagents and waste in compliance with regulations in force.
- Read all the instructions provided with the kit before running the assay.
- Follow the instructions provided with the kit while running the assay.
- Do not use the kit after the expiry date.
- Only use the reagents provided in the kit and those recommended by the manufacturer.
- Do not mix reagents from different batches.
- Do not use reagents from other manufacturer's kit.

### Warnings and precautions for molecular biology

- Molecular biology procedures, such as extraction, reverse transcription, amplification and detection of nucleic acids, require qualified staff to prevent the risk of erroneous results, especially due to degradation of the nucleic acids contained in the samples or due to sample contamination by amplification products.
- It is necessary to have separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions.



- It is necessary to have lab coats, gloves and tools which are exclusively employed in the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never transfer lab coats, gloves or tools from the area designed for the amplification/detection of amplification products to the area designed for the extraction/preparation of the amplification reactions.
- The samples must be exclusively employed for this type of analysis. Samples must be handled under a laminar safety box. Tubes containing different samples must be never opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.
- Reagents must be handled under PCR box or laminar flow box. The reagents required for amplification must be prepared in such a way that they can be used in a single session. The pipettes employed to handle the reagents must be used exclusively for this purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.
- Amplification products must be handled in such way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be employed exclusively for this specific purpose.

#### **Warnings and precautions specific to components of the kit**

- The tubes containing mix (C. pneumoniae UNI Mix) are disposable and therefore must be used once only in the preparation of the reaction mixture.
- The tubes containing IC DNA 01 and PC DNA C. pneumoniae are disposable and therefore must be used once only in the preparation of the reaction mixture.
- These mixes carry the following safety warnings (P):

**P280** Wear protective gloves/protective clothing/eye protection/face protection.

**P281** Use personal protective equipment as required.

#### **Literature**

Pignanelli S, Shurdi A, Delucca F, et al. 2009. Simultaneous use of direct and indirect diagnostic techniques in atypical respiratory infections from Chlamydia pneumoniae and Mycoplasma pneumoniae. J Clin Lab Anal. 23 (4): 206–9.

Roulis E, Polkinghorne A, Timms P. 2013. Chlamydia pneumoniae: modern insights into an ancient pathogen. Trends Microbiol. 21(3):120-8.



## Symbols



Catalog number



Upper limit of temperature



Batch code



Use by (last day of month)



*in vitro* diagnostic medical device



Fulfilling the requirements of European Directive 98\79\EC for *in vitro* diagnostic medical device.



Contains sufficient for "N" tests



Attention, consult instructions for use



Manufacturer

## Manufacturer

**ELISABETH PHARMACON Ltd.**

Rokycanova 4437/5, 615 00 Brno, Czech Republic Tel.: +420 542 213 851, +420 542 213 827

E-mail: [info@elisabeth.cz](mailto:info@elisabeth.cz)